# The validity of the Bunsen-Roscoe law in the production of mutations by radiation of extremely low intensity.

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## Gene mutations.

# INTRODUCTION.

The gene is regarded as a unit having a definite chemical and space configuration which remains perfectly constant except during mutation. The fundamental problems in genetics are - how do mutations arise and what is the nature of these units ?

X-ray genetics, started since Muller's discovery of the artificial transmutation of the gene by X-rays, has provided us with an excellent tool to attack these problems from different angles. One of the different lines of attack has been the quantitative determination of the mutation rates under varied conditions of radiation. The most outstanding results obtained are :-

(a) the rate of gene mutation is directly proportional to the dosage when only the duration of treatment is varied. (Hanson & Heys, 1929a; Oliver, 1930; Timofeeff-Ressovsky, 1934a, etc.). (For variation of intensity and fractionation see discussion of Bunsen-Roscoe law.)

(b) the mutation frequency is independent of the energy content of the individual quanta of radiation over the range of very soft X-rays (Grenz-strahlen) to gamma rays when the same dosage in r units is given.

(Schechtman, 1930; Efroimson, 1931; Hanson, Heys and Stanton 1931; Gowen and Gay, 1933; Timofeeff-Ressovsky, 1934c; Pickhan, 1935; Fricke and Demerec, 1937.).

(c) the temperature coefficient of the induced mutation rate is unity. (Timofeeff-Ressovsky, 1934b; Makhijani, 1939; unpublished.)

Bunsen-Roscoe law and biological reaction.

The product of the time of irradiation and intensity per unit time gives the X-ray dosage. The Bunsen-Roscoe law (when applied to biological re-:actions) can be stated thus - the effect is always the same regardless of the variation in the intensity or the time of irradiation without modifying the product on the conidtion that a compensation of the variation of the one by an inverse variation of the other be made.

This law holds true for many ordinary physico-chemical reactions over a very wide range of intensi-:ties. It is a result of the individual quanta of the energy as finally absorbed producing their effects indipendently of one another. Thus, when applied to biological reactions it is expected to give us some idea about the nature of the reaction.

## Physical basis of X-ray action on living material.

An accurate mental picture of the different processes involved during radiation is essential for a biologist when he studies the effect of these rays on living substance. The sequence of events following radiation may be assumed to be (a) ionization (b) primary chemical changes (c) mutation or other biological reaction. Two distinct processes are constantly going on in the substance through which radiation is passing, viz., ionizations and recombinations.

Ionizations: Radiation is a form of energy, which, according to current physical theories, is produced in measurable discrete quanta. Part of the energy always passes through the material under radiation without losing any energy and evidently cannot produce any change in the material. It is only in cases when a quantum is absorbed (directly by photoelectric effect or indirectly by Compton effect) by an atom, that it causes an electron to leave the atom with huge velocity pushing out other electrons which happen to be present in its path. The released electrons produced in the process get themselves attached to neutral atoms nearby, and form so-called "negative ions" due to the addition of an extra electron. The

atoms which lose the electrons are the "positive ions." Thus ions are always produced in pairs, and the con-:centrations of the positive and the negative ions are always the same at any given time and given dosage. When an atom is ionized it may no longer be able to occupy the same stable position in the molecule as it did formerly and so a chemical change in the molecule may be favoured. Moreover, it may transfer its charge to another atom, and so on, in a chain, until a chemically unstable configuration has been reached. Another possibility is for the atom to be torn bodily from a molecule, thus making the latter reactive.

Recombinations: The ions of opposite polarities eventually come together primarily by atomic and mole-culer collisions where thermal energy is expected to play an important part. The results of all these chance encounters too are, that some atoms or mole-:cules are brought together under conditions which favour a re-grouping of the atoms and consequently chemical changes are expected to take place.

The above mentioned processes are constantly going on in the substance through which radiation is passing. It is possible that the ensuing biological reaction might be dependent not only on the number of ionizations but also on the rate of recombination,

and so on, any factors conditioning the latter (Failla, 1936).

Most of the experiments dealing with other biological reactions depart from the Bunsen-Roscoe law by showing lesser effectiveness of a given dosage of radiation at lower intensities. Below, we quote a few examples in a tabular form (Table I. modified after Griffith & Zimmer 1935) to show the diversity of materials used and the results obtained. The nature of the time-intensity factor in relation to radiation and consequent biological reaction can be expressed mathematically thus :

$$B = I^n \; T^p$$

where, B = biological effect,

I = intensity per unit time,

T = time of irradiation.

The above formula becomes the Bunsen-Roscoe law only when $n = p$ (i.e. $\frac{n}{p} = 1$).

The results of the different workers have been discussed and summarised by Pack and Quimby (1932, Griffith and Zimmer, 1935), and Scott(1937). Scott has drawn the following conclusions from the results discussed by him. "In general the intensity of an irradiation influences its biological action in the

TABLE I.

Time-intensity factor in relation to biological reaction (modified after Griffith and Zimmer, 1935).

| Author | Material | Index of reaction | Value of $\frac{n}{p}$ | Range of intensity |
|---|---|---|---|---|
| 1. Lasser (1930) | Tissue Culture | growth rate | =1 | 1:18 |
| 2. Packard (1926) | Drosophila eggs | death | =1 | 1:4 |
| 3. Packard (1928) | " | " | >1 | 1:10 |
| 4. Sievet and Forssberg (1931) | " | " | =1 | 1:900 |
| 5. Leichti (1929) | Daphnia | " | >1 | 1:10 |
| 6. Holthusen (1933) | Human skin | ulceration | >1 | - |
| 7. " | " | erythema | >1 | - |
| 8. " | " | epilation | >1 | - |

following way: over the range of relatively feeble intensities, the biological effect is rapidly increased as the intensity increases, but there is a critical value above which the effect of radiation is independent of its intensity." Under the present state of knowledge, such a conclusion can be accepted only when the reaction which is taken as the biologi-:cal measure is other than mutation. Of course, it is only at comparatively low intensities that the value of n/p in ordinary biological reaction is greater than unity. In genetic experiments with X-rays, we find that the lowest intensity so far used is 1r/min. (Timeofeeff-Ressovsky and Zimmer, 1935). In defining the nature of the curve of mutation frequency in relation to intensity of radiation, which is a straight horizontal line (parallel to intensity), the gap between the control value and the point obtained at the lowest intensity is very important because it might be expected that the linear relationship may not hold true at even lower intensities and there may be a rapid falling off in the effect at very feeble intensities. If so, the inference mentioned below relating to some fundamental ideas of mutation process would have to be changed. As before noted, studies on the effect of

different wave-lengths of radiation on the gene mutation process have shown that, within a wide range, the mutation frequency is sensibly independ-:ent of the space distribution, i.e. of the local concentration, of the ions. These studies do not, however, differentiate between the two phases of the chemical changes, viz. (1) the extremely rapid primary reactions which are induced by the initial activations, and (2) slower secondary reactions induced by the primary changes involving the whole medium in general (Fricke and Demerec 1937). Since the latter would involve a kind of spreading effect, it is the opinion of Scott and other competent workers in radiology, that it cannot be concluded with certainity from this result alone that the mutations are consequences of individual ionizations acting quite independently of one another. Due to the above considerations experiments to test the mutation frequency at extremely low intensity are very im-:portant. It would be desirable at the same time to have them performed under conditions giving the minimum chance for unequal space and time distribu-:tion and consequent local concentration of ions which would conceivably form the spreading or joint

action of ion. Such conditions being presented by gamma as opposed to X-rays. Even on the very simple assumptions of mutation being dependent on changes in the chemical constitution of the medium in general, the amount of effect might well depend on the rate of production of ions, and it is almost certain that if mutation is dependent on slower secondary re-:actions, the rate of diffusion of the altered sub-:stances from the primary loci of change outwards, must play some part in the mutation frequency, and the effect of this factor would be expected to vary with the rate of ionization at low intensities. Thus, further to develop any theory of 'direct hit' or otherwise, a test at low intensity might by very helpful.

Time - intensity factor in relation to gene mutation.

Researches on the relation of the time distri-:bution of the ionization to the frequency of the gene mutation have been done mainly on Drosophila. Patterson (1931) has given a dosage of 1200r in 10 min. and again applied the same dosage (at the same intensity) in 8 parts with various time intervals and finds no significant difference in the percentage of mutations in the different groups.

Timofeef-Ressovsky, 1934c, Timofeef, etc.

Zimmer and Delbrück (1935) obtained similar results, showing the frequency of the mutations to be independent of the "fractionation" of the dose into as many as six parts. Researches of this nature where the dosage applied in a rather small number of parts cannot really test the validity of the Bunsen-Roscoe law in view of the fact that each part may be assumed to have given the amount of energy needed for a linear relation of frequency to dosage. They have also tried the effect of changing the intensity of the irradiation, compensating for this by inverse changes of the duration of treatment. In these experiments they used intensities ranging between 300 r/min. and 1r/min. and obtained no significant difference. Hanson and Heys (1932) applied a series of dosages as exactly equivalent as possible in different ways, that is, in one case high intensity over a brief period as compared with an equivalent dose of low intensity spread over a longer period. The range of intensities here was 1: 150. They found no difference due to the distribution of the dosage. It may be remarked, however, that the percentages of mutation found by them at the different dosages specified are widely different from those obtained by any other

worker working on mutation in Drosophila sperm.

The present investigation was undertaken to test the validity of the Bunsen-Roscoe law by studying the frequency of sex-linked lethal mutations and translocations in Drosophila melanogaster at much lower intensities of radiation than those previously tried. The results on translocation are dealt with in part II.

List of flies used in the experiment.

1. Samarkand stock -wild type flies.

2. sc v C1B / sc v f car — One of their X chromosomes contains an inversion (C), which virtually supresses crossing over throughout the entire chromosome, a dominant mutant gene Bar (B) and three recessive mutant genes: scute (sc) vermilion (v) and lethal (l). The homologous X chromosome contains four recessive mutant genes: scute (sc), vermilion (v), forked (f) and carnation (car).

3. Cy D males. - A synthesised heterozygous stock having in one of the second and one of the third chromosomes the dominant marker genes Curly (Cy) and Dichaete (D), respectively. Both of these marked chromosomes contain inversions which greatly reduce the number of crossovers obtained. The homologous second and third chromosomes contain a group of recessive mutant genes familiarly

called 'apl' and 'rucuca', respectively.

4. $\frac{\text{sc dl 49 v f}}{\text{sc dl 49 v f}}$ - A X-chromosome stock homozygous for the three recessive mutant genes indicated, and an inversion (dl 49).

5. $\frac{\text{bw}}{\text{bw}}$ $\frac{\text{e}}{\text{e}}$ $\frac{\text{ey}}{\text{ey}}$ - Stock containing brown (bw), ebony (e), eyeless (ey) recessive mutant genes in their second, third and fourth chromosomes, respectively, in homozygous condition.

6. $\underline{\text{Sc8 B w}^{a}}$ - Stock having the mutant genes Scute 8, Bar and apricot ($w^{a}$) in one of their X chromosomes, and a long inversion, associated with Scute 8.

## Stage to be treated.

While outlining the general plan of the experiment it was decided to treat the flies with gamma rays of radium for 720 hours (30 days) continuously, giving a dosage of as low intensity per minute as feasible, in view of the long duration of treatment. Mature Spermatozoa were chosen as the stage to be irradiated. There were two reasons for this: First, it had been shown (Harris, 1929; Hanson and Heys, 1929b; Muller, 1928, 1930; Siderov, 1931; Moore, 1934, etc.) that the frequency of gene mutations and also of translocations is significantly lower in the immature germ cells than in the mature sperms. Second, only in the case of these cells could the material be held a sufficiently long time in the same stage. But to achieve the latter object it was thought essential to treat mature sperms in the body of the females rather than to irradiate the males. We do not know yet for certain about the duration of retention of the mature spermatozoa in the males while kept for such a long time without females. By treating the males we not only treat the mature sperms but also the immature germ cells in the testes. The treated

males in that case, after the expiry of a month could possibly have fertilised the females with sperms derived from cells which were immature at the time of irradiation. A comparison with other groups in which the males receive radiation at higher intensities and consequently during a much shorter period, and were bred soon afterwards, would therefore be open to objection, since in the latter cases the sperm used would have been mature at the time of irradiation.

The experimental difficulties in treating inseminated females are the following: (a) they are comparatively very old after the month's treatment and consequently lay, on an average, much lesser number of eggs. This is a great disadvantage because it is essential to have a large number of progeny for a significant result, in view of the low number of mutations expected at a low dosage; (b) the question of the retention of the sperms in the ventral receptacles of the females after insemination brings in another difficulty. Under the usual conditions of temperature and food, the sperms are not retained in the body of the

females, being used up in the process of egg laying. To overcome both these difficulties partially, a test experiment was carried out on the retention of the sperms in the body of the females under different conditions of temperature and food.

Test experiment.

Virgin females of the wild Samarkand stock were collected and kept with an equal number of young males of the same stock for 72 hours in a constant temperature room (23°C) to secure insemination. 60 males and 60 females were put in a culture bottle for the purpose. After this period the males were separated and the inseminated females were divided into following groups:

A. Flies to be kept on syrup food ( D.I.S.) at 8°C.

B. Flies to be kept on syrup food at room temperature

C. Flies to be kept on Offermann's yeast food (D.I.S.) at 8° C.

D. The inseminated females to be X-rayed with a dose of cal500r and then to be kept at 8°C. on syrup food.

The flies were kept under the conditions specified, in small vials for 720 hours. The

TABLE II.

Test experiment on the retention of sperms.

| Groups | Number of flies tested after 720 hours | Number of eggs laid | Number of larvae obtained | % of fertility | Reproduction rate |
|---|---|---|---|---|---|
| (A) | 13 | 3186 | 1908 | 59.5 | 146.7 |
| (B) | 20 | 2271 | 1309 | 57.6 | 65.4 |
| (C) | 19 | 725 | 201 | 27.2 | 10.5 |
| (D) | 14 | 486 | 53 | 10.9 | 3.08 |

fertility of the different groups of flies was tested at the end of the period. The results are shown in Table II.

The results obtained show that the performance of the flies of the A group was best. The reproduction rate gave some idea about the number to be treated in the main experiment in order to obtain the required number of F1 flies.

## Method for detecting sex-linked mutations and translocations.

The following methods were those at first used for detecting translocations and sex-linked lethal mutations at the same time instead of using a separate system of crossing for each test. Cy D males were allowed to inseminate ClB virgin females. The inseminated females were then rayed for 720 hours while on syrup food at 8°C before allowing them actually to breed. The viable F1 flies of the above cross were of twelve kinds because of the independent segregation of the marked chromosomes. Half of the males die due to the lethal gene in the ClB chromosomes; this produces a sex ratio of 2 females to 1 male. The F1 females of the above cross having the following constitution $\frac{ClB}{+}\ \frac{+}{Cy}\ \frac{+}{D}$ (the chromosomes from the Cy D males are marked with heavy lines) were collected every 12 hours, because it is essential to have them virgin for detecting translocations between the Curly and Dichaete chromosomes. The F1 virgins of the above constitution were bred separately in small vials having one male

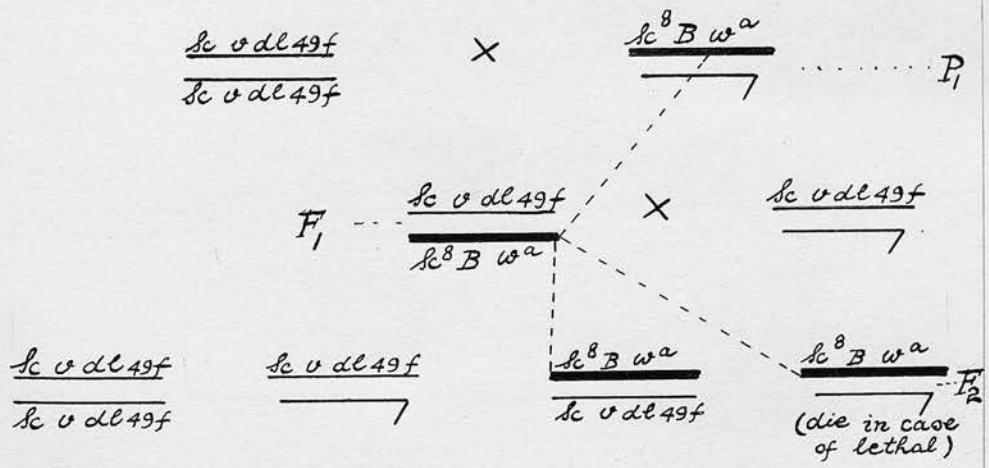
and one female in each vial. The males used for these pair matings were "scar" males (scute, vermilion, forked, carnation). Each of these vials should normally produce 16 kinds of zygotes, of which twelve are viable, the sex ratio of each family being 2 to 1 again, due to the lethal gene already present. But if a lethal mutation has occurred in the treated X-chromosome of the sperm the other half of the males also will die. Thus sex-linked lethals were detected by observing the vials which produced no males at all, or none except non-disjunctional males. Occasionally one or two non-disjunctional males appeared in the vials to be scored as lethals but these were easily recognised because they had received their X-chromosome from their father and this was marked with the four recessive characters of the "scar" combination. For the detection of lethals alone all the F1 Bar females could be used. Virginity of these females was not necessary for this purpose.

The other markers - Curly and Dichaete - were intended for the detection of translocations simultaneously with the lethals, as will be explained

in the parallel paper on translocations. As their presence did not in any way change the method of transmission of the above sex-linked lethals on the above scheme, the details concerning them need not be entered into here. Considerable trial was made of the above genetic method, and reliable results concerning sex-linked lethal frequency were obtained, which are given in the present report (Series 1 - 5, inclusive). It was decided, however, to use a different method in the later series, as it was found impracticable to use the same F1 flies in these experiments for the purpose of detecting both lethals and translocations. This was because the requisite numbers of F1 females of the right kind for such testing were too difficult to obtain, since for the translocations and not for the lethals on this method these females had to be virgin, and not merely Bar but at the same time Curly and Dichaete (as will be explained). In the later series, then, the technique was changed, and separate flies were used for the detection of mutations and of translocations, although they were irradiated simultaneously. When this separation of the work was made, it was desired to use methods which would

provide as large as possible a number of F1 suitable for testing. This was particularly desirable because of the fact that the number of $P_1$ treated flies available for either mutation or translocation was now diminished by their separation into two different groups. Accordingly, it was decided not to use ClB females in the P1 generation, since, being heterozygous, they give daughters only half of which are of the desired composition for the tests. Instead a homozygous stock of composition Sc v dl 49 f was used. This contains in the X chromosome, besides the recessive genes indicated, the inversion "dl 49" which is of moderate length. These females were crossed to males of the stock containing the sex-linked genes Sc8 B $w^a$. Here there is a very long inversion associated with the Scute 8 character. All the regular females from this cross are alike and can be used for the tests. The heterozygous presence of both the long and moderate sized inversions very effectively prevents the production of crossovers by such females. In using this method normal appearing $F_1$ females were collected and mated individually to their Sc v f brothers in separate

vials. Lethals that had been produced in the X chromosome of the sperm were detected by the absence of Sc8 B $w^a$ males. The scheme of crossing is shown below. The treated X-chromosome is shown with a heavy line and its transmission indicated by a dotted line.



## The experimental set-up.

Fig. I is an isometric view of the wooden box in which the flies were kept during radiation and table III shows the different groups of experimental flies. Total doses of 400 and 2000r were given to the low treated and high treated groups, respectively. Since each milligram of radium is known to give 1/7.5r per minute at a distance of 1 cm., it is evident that 70 mg. at the distance of 14.2 cm. will give 0.045r per minute or ca 2000r in 720 hours (1 month). At a distance of 31.8 cm. it will give ca 400r in 720 hours.

Disposition of the radium: 70 mgms. of radium contained in 54 platinum needles (50 one mgm and 4 five mgm) were placed in a dish shaped depression cut into the centre of the top of a cubical wooden box (see Fig. I). The 5 mgm. needles were arranged in the centre and the 1 mgm. ones placed nearer to the sides of the groove. They were crowded together as closely as possible while leaving them in the same plane. The diameter of the place occupied by the needles were 5 cm. (the arrangement is shown in fig. II).

Dishes for the treatment of the flies :

The flies were treated in round bakelite dishes each provided with a transparent lid of celluloid calm.

TABLE III.

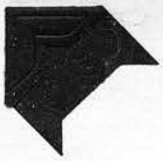
(Showing the different groups of experimental flies).

| Groups | Time of irradiation | Distance from the radium | Calculated dosage | Intensity |
|---|---|---|---|---|
| A. High treated | 720 hrs. | 14.2 cm. | 2000r | cal/20r min. |
| B. Low treated | 720 hrs. | 31.8 cm. | 400r | cal/100r min. |
| C. Controls | (kept for 720 hrs. before breeding) | - | - | - |
| D. Concentrated (2000r) | 45 hrs. | 3.5 cm. | 2000r | ca4/5th.r min. |
| E. Concentrated (400r) | 9 hrs. | 3.5 cm. | 400r | " |

thick having a few small perforations. Their exact size is shown in fig. II.

High treated group:

The upper surface of the upper shelf was at 16. 1 cm from the radium. The desired distance for the high treated group was calculated to be 14. 2 cm. and therefore the flies to be at that distance should be 1.9 cm. higher than the upper shelf. As the dish is 2.6 cm. in external height, with the food one cm. below the top, the surface of the food, on which at the temperature of 8°C the flies rest nearly all the time, is 1.3 cm above the lower surface of the dish. Thus the dishes needed to be raised 0.3 cm. Allowing, however, for their positions slightly to the side of the veritcal line drawn from the centre of the radium to the shelf the dishes were tilted so as to face the radium, by means of supports 1cm height placed under that part of them which would have been furthest from the radium. The portion of the dish near the middle of the box rested on the upper shelf (see Fig III). It will be seen that the error in distance could not have been more than a few millimeters. Variation of distance due to the fact that



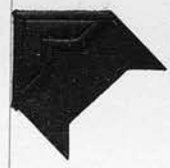
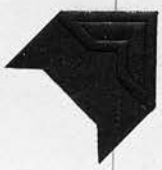

both flies and radium were spread out (practically evenly) over a circular surface about 7.5x2 and 5 cm, respectively, in diameter, likewise could not have introduced a significant error according to the calculations of Dr. Murison (see below).

Low treated group:

The distance of the flies from the radium was to be at 31.8 cm. The bottom of the box (upper surface) is 40.5 cm from the radium. Hence, considering the height of the food above the bottom of the dishes, they were supported on a smaller wooden box 7.1 cm high, resting on the bottom of the main box (see Fig. III).

Concentrated group (ca 2000r):

The distance of the plane of the flies from the plane of the radium was 3.5 cm. The flies were placed on a wooden block 11.6 cm high placed on the upper shelf (allowing 1 cm for food plus the height of the projection of the dish at the bottom (0.3 cm). The arrangement is shown in fig. 3. The distance is as nearly as possible one fourth of the original distance (although necessarily with a greater error) of the "high treated group" so that the intensity is 16 times

as high. The time of radiation was therefore reduced correspondingly to 45 hours.

Concentrated group (400r):

The flies were kept at exactly the same distance as the above. The time of exposure was 9 hours.

Controls:

The control flies were handled exactly in the same way except for radiation being placed in dishes, and these within boxes of just the same type. After insemination the Control females were kept on syrup food at 8°C for 30 days before breeding.

Cooling procedure during the period of irradiation:

The whole box was placed in a Hearson's patent refrigerator which was kept running at a temperature of 8°C with an occasional rise of 2 to 3°C for brief periods. The low temperature was maintained by putting ice in the ice chamber of the refrigerator which had a thermostat control. During the later part of the work (series 5 - 8, inclusive) the box containing the Controls was kept in another refrigerator of the same type. In the earlier part of the work the

wooden box in which the Controls were kept was placed in a large refrigerator, and warmed to approximately 8°C .

Thanks are due to Dr. Murison, physicist to the radiological department of the Royal Infirmary, Edinburgh, for his examination of the whole set-up and advice concerning it.

Description of a typical experiment.

3600 C1B virgin females were collected from 96 culture bottles during a period of 7 days. The same number of Cy D males were also collected from 24 bottles at the same time. Daily collections of virgins were stored in small bottles containing syrup food in a refrigerator running at 8 to 10°C to reduce their metabolic activity. After the required number of flies were obtained the females were put into 48 bottles of Offermann's yeast food, about 70 in each bottle. Approximately equal numbers of Cy D males were also put in the bottles for inseminating the females. The males and the females together were then kept in a constant-temperature room of 23°C for 72 hours. At the end of this period the sexes were separated and the males were discarded. The females were all mixed together and then divided at random into three lots of 1000 each for the three main groups (high treated, low treated and controls). For radiation as well as for the controls, two dishes of syrup food were used for each group with 500 flies in each dish. The surface of the food was sprinkled with white crepe paper confetti. These dishes were put into the irradiation and control boxes, respectively,

in the two cooled refrigerators, in the positions already described.

The flies were taken out of the cooled boxes after exactly 720 hours, and were allowed to breed in half pint bottles, having the standard amount of Offermann's yeast food. The description of the number of flies put in each bottle and other relevant information are given in table IV.

The $P_1$ - $F_1$ culture bottles were kept in the 23°C constant-temperature room for breeding for 10 days, the female being discarded after 7 days. In the first five series $F_1$ virgin females of the following types, viz: B Cy D, B Cy, B D and B were collected twice daily at approximately 9 a.m. and 9 p.m. In the later series in which the flies for mutation and translocation were separated, virgins were unnecessary as previously noted. During the collection of $F_1$ the bottles were continuously kept at the room temperature (12-18°C). Collection of $F_1$ virgins (in the first 5 series) was made for about 7 days. Pair matings of the $F_1$ females were done in vials simultaneously. The vials were kept in the 23°C Constant temperature room for about 12 days

TABLE IV.

| Group | No of flies kept for period of irradiation | No of survivals after period of radiation (720 hrs.) | Bottle cultures made | Flies per bottle for breeding | F1 females used for pair matings | Fertile $F_2$ cultures |
|---|---|---|---|---|---|---|
| High treated | 1000 | 621 | 10 | 60 | 162 | 122 |
| Low treated | 1000 | 642 | 20 | 30 | 812 | 525 |
| Controls | 1000 | 592 | 20 | 30 | 802 | 604 |

N.B. The numbers in the above table are taken from the third series of the experiment, when the breeding methods had become standardised.

In series VI, VII and VIII there were about 700 flies in each group kept for the period of irradiation. Half of them were bw e ey females inseminated by Samarkand males for translocations and the other half were Sc v dl 49 f females inseminated by Sc8 B $w^a$ males for lethals. There was no Control group for translocations. The number of $F_1$ flies used for pair matings was similar to that of first five series.

before they were examined. Each actual or suspected lethal and translocation obtained in the experiments was tested for one more generation. Only the results obtained from these $F_3$ confirmations were scored as lethals and translocations.

## Results.

The whole procedure just described was repeated eleven times at approximately monthly intervals between the start of each series and of the next one, so that the incubators were kept in maximum use continuously.

Unfortunately, three of these eleven series failed to give any progeny in the $F_1$. This was due to the use in these series of a different stock which had been prepared for detecting translocations and mutation at the same time by means of recessive markers. It had been hoped in this way to obtain a larger number of $F_1$ females of the appropriate type, since the stock was homozygous for its autosomal markers. The genetic make up of this stock is given below :-

$$\frac{\text{Sc v ClB}}{\text{Sc v dl 49 f}} \qquad \frac{\text{dp}}{\text{dp}} \qquad \frac{\text{e}}{\text{e}}$$

Virgin females of the above stock, impregnated with males of the Samarkand wild type stock were kept under radiation. During the period of radiation most of the flies died. The rest that survived,

when cultured in bottles with approximately the same number per bottle as used previously, gave practically no progeny. The failure of this stock is in all probability due in the main to the effect of the mutant dumpy (dp).

The results of the remaining eight series of experiments are given in the table below (Table V). 3471 fertile $F_2$ cultures were examined, in all the control series added together, and 11 lethal mutations were obtained: this gives a percentage of $0.317 \pm 0.095$. The variation in numbers between different control series were not significant, as even the total number of spontaneous sex-linked lethal is quite low however it has fairly high standard error. If we compare this figure with figures obtained by different workers we find the present value to be a little higher than those oftenest obtained, yet not higher than values obtained quite commonly (see for instance Muller, 1928 and Demerec, 1937). The control value in the present experiment is very important because of the low number of the mutations expected in the low treated group. Every care was taken to handle the control group in exactly the same way as the treated groups, except for radiation. The control flies were kept

TABLE V.

(Showing the results of each series separately.)

| Series | Controls | | Low treated ca400r | | High treated ca2000r | |
|---|---|---|---|---|---|---|
| | Tested $F_1$ - $F_2$ cultures | No of lethals obtained | Tested $F_2$ - $F_2$ cultures | No. of lethals obtained | Tested $F_1$ - $F_2$ cultures | No. of lethals obtained |
| I | 520 | 2 | 510 | 9 | 102 | 7 |
| II | 435 | 2 | 436 | 9 | 80 | 7 |
| III | 604 | 3 | 525 | 8 | 122 | 10 |
| IV | -- | - | 593 | 9 | 121 | 4 |
| V | 537 | 2 | 543 | 7 | 121 | 10 |
| VI | 432 | 1 | 379 | 4 | 99 | 6 |
| VII | 632 | 1 | 457 | 6 | 96 | 7 |
| VIII | 311 | 0 | 412 | 7 | 127 | 7 |
| Total | 3471 | 11 | 3855 | 59 | 868 | 58 |
| % of Lethals | 0.317±0.095 | | 1.53±0.1995 | | 6.675±0.846 | |

in another refrigerator running at the same terperature, and approximately with the same variation of temperatures as the other two groups. Unfortunately, the control flies in the IVth series were found dead after the period of one month. This was due to an accidental drop of the temperature of the refrigerator to about 0°C because the thermostatic control went out of order.

Beginning with Series VI flies of the stock Sc8 B $w^a$ were used as controls. It will be seen that they gave sensibly the same mutation rate as the others.

The low treated group with ca400r gave 59 lethals in 3885 X chromosomes examined. There is no significant variation between the different series. The percentage obtained is 1.53 ± 0.1995. The percentage of the lethals obtained which were due to treatment is calculated by deducting the control value, this leaves a percentage of 1.213 ± 0.222, which is in as close agreement as expected with the results obtained by Oliver (1930), Timeofeeff-Ressovsky (1934a) and others with X-rays as well as with radium. The $E_{L-C}$ (error of the percentage due to treatment)

is here taken as $\sqrt{E_L^2 + E_C^2}$ where L and C are the values of low treated and control series, respectively. 868 X chromosomes were analysed in the high treated group and 58 lethals were obtained or 6.675% ± 0.846%. Again there was no significant difference between the results of different experiments. The mean percentage due to treatment with ca2000r is 6.35 ±0.852.

In the present experiment the factor varied was the intensity of radiation per unit of time, everything else remaining constant. The intensity of the high treated group was five times as high as that of the low treated group and consequently the total dosage obtained ( ca2000r) was also five times that of the low treated series. A way of making the comparison in these two groups is to multiply the mutations due to treatment in the low series by five. We then get a percentage of 6.065 ± 1.105 (expected percentage on proportionality basis) which is to be compared with that of 6.35±0.852% for the high series. The difference is 0.2851 ± 1.395 which is even less than its own standard error and therefore quite insignificant (see table VI).

TABLE VI .

(Comparing the high treated with low treated group).

| Group | % Lethal | due to treatment | Expected % on proportionality basis | Difference F - D | Difference / E. Difference |
|---|---|---|---|---|---|
| Control | 0.317±0.095 | -- | ----- - -- | | |
| Low treated | 1.53 ± 0.1995 | 1.213±0.222 | ---- - -- | 0.2851±1.395 | 0.204 |
| High treated | 6.675±0.846 | 6.35 ± 0.852 (F) | 6.065±1.105 (D) | | |

TABLE VII.

Comparing Concentrated (2000r) group with High treated (2000r).

| Group | % Lethal | Difference |
|---|---|---|
| Concentrated 2000r | 5.25 ± 0.821 | 1.425 ± 1.18 |
| High treated 2000r | 6.675±0.846 | |

The results obtained thus agree with the conception of a direct proportionality between the dosage and the mutation rate, independently of the intensity, even with an intensity as low as 1/100r/min.

Another experiment was undertaken to compare these results with those of a total dosage of 2000r in 45 hours. The latter represents an intensity 16 times as high as that of the corresponding high treated group in the previous series. This intensity ( ca4/5r per min.) was very nearly as high as the lowest intensity (1r/min) used by Timofeeff and Zimmer (1935) which was the lowest intensity previously investigated. The percentage of lethals was 5.25 ± 0.821 in 762 X chromsomes studied. (91) This is a little low when compared with the other group, though it is still within the limits of statistical error, especially when the greater error of the distance in this case is taken into account (see table VII).

If we examine the graph (Fig. IV) which is obtained by plotting the frequency of mutations per 100r against intensity of radiation (represented

logarithmically), we find that the curve is a straight horizontal line. That is, the frequency for a given dose is constant, regardless of intensity. The 3 points at very low intensities were obtained from the results of this experiment while the other points were obtained from the data of different authors (Oliver, 1930; Timeofeeff-Ressovsky, 1937). A glance at the graph shows that there is no tendency to falling off of the curve even at such low intensities as those here used. These are almost the lowest with which an experiment of sufficient size to give significant results can be conducted with this material.

The present experiment then, taken together with the previous works on proportionality, shows that the Bunsen-Roscoe law holds in the production of mutations by radiation within the very wide limits of intensity of 0.01r/min (low treated group) and 300 r/min. (Timofeeff-Ressovsky and Zimmer (1935), a ratio of 1 to 30,000. The time factor varied from 5 min.to 720 hours, a ratio of 1 to 8640.

## Discussion and conclusions.

Below is shown the time distribution of the ionizations in the irradiated sperms in the present experiments. This is calculated by taking into account the fact that 1r unit gives $4 \times 10^{12}$ ion pairs per cubic centimetre of organic substance and that the volume of the Drosophila sperm head is $1 \times 10^{-12}$ $cm^3$. The ionization effect produced in the low treated group is, of course, directly proportional to the dosage applied at higher intensities.

Low treated - 2.2 ion pairs per sperm per hour

High treated - 11 " " " " " "

Concentrated (2000r) 176 " " " " " "

From this result under these conditions of time and space distribution of ions it is reasonable to conclude that individual ionizations, acting within a rather limited space, cause the necessary changes for the production of mutation. In other words, we have in an individual mutation a reaction in which we observe the effect of one single "encounter" between a quantum released by the radiation and the genetic material. Neither the distribution of ions

in space within wide limits (studies on wave length) nor in time (results of present investigation) has any differential effect on the mutation process. With an individual ion, no matter whether or not it is associated with other ions, there is a certain definite chance of a mutation being produced. These minute definite chances summate exactly in time to produce the observed frequency. The relatively slower secondary effects of radiation, by way of diffusion of altered chemical substances from the ionization loci and interaction of those derived from different ionizations with one another, have no detectable effect on the mutation frequency.

Three factors might alter the validity of the Bunsen-Roscoe law in relation to mutations: (a) a restitution process affected by ion concentration, (b) a change in radia-sensitivity thus affected, or resulting from any other factor during the time of treatment, and (c) (in a sense a special case of b) the existence of a threshold intensity, owing to the action of more than one ion being required in a given space and time. In regard to (a), it should be noted that mutation is not readily

reversible, although the gene may go from one stable condition back to another, the interphase is of very high order. Experiments to test whether there is any natural restitution of X-ray effect within the treated sperm themselves have been carried out by Muller (1928), and confirmed and extended by Harris (1929). Irradiated males were divided into different lots and bred at different time intervals and the mutation rate tested. The males used in aging were kept without females before they were allowed to breed. They found no significant difference in the mutation rate in different groups, showing that there is no back reaction here appreciable.

Examining the question (b) of a possible change in the radio sensitivity of sperm, Timeofeeff-Ressovsky (1931) treated young and old males with identical doses of X-rays to test whether any change at all occurs in the mature sperm, no matter how long they are aged. Unlike the experiments of Muller and Harris he irradiated the males after the process of aging, which was also done in the same way by keeping the males without females for 15 to 20 days.

His experiments likewise gave negative results. Offermann (1939), however, reports the finding of a higher mutation rate in older sperms. In finding the time distribution of the age effects he tested the frequency of induced lethals in sperm aged in females during 0, 12, 24, 36 days and found that they are not time proportional, since he found a significant increase only at the last period. This result seems at first to have a direct bearing upon our problem when we compare the concentrated with the diluted group. In the diluted group the sperms are more than a month old (of course receiving radiation all the time) while in the concentrated group they are only a few days old. We did not, however, find any significant difference of the mutation rate in the two groups. This might be partly due to the fact that the sperms in the diluted series did not receive the total amount of radiation at the old age. But it would mainly be because our lots, unlike Offermann's, were kept at the low temperature of 8°C so that the physiological aging must have been much less than in his flies. The results of Offermann do not in any reasonable way affect the validity of the comparison of our two groups.

Offermann does not find any such difference in the mutation rates in the eggs, a fact which he explains might be due to the continuous renewal of the eggs in the females. Renewal of sperms in the testes is not inconceivable and therefore the comparison which Timefeeff-Ressovsky made when he aged the sperms in the males is open to objection. These considerations at least justify the trouble taken in the present experiment to treat the inseminated females.

The existence of a threshold intensity (c) below which no mutation can be produced is, of course, directly in contradiction with the results obtained in the present experiment, unless this intensity is set a good deal lower than .01r/min (in view of no change in the curve being perceptible in this region of it). It should be taken into consideration that in our low series there was, on the average, but one ion pair produced in a sperm every 27 minutes. It would seem very far fetched to assume that a single ion pair could not produce a mutation, but could nevertheless have its effect stored up for hours, and then so interact with the effects of the later ion pairs as to give a frequency of mutations exactly

proportional to the total number of ion pairs. This becomes still more cogent when we consider the probable spatial limitation of the effects of an individual ion pair. On the basis of these considerations and of the present data then, the applicability of the Bunsen-Roscoe law to the mutation reaction is held to be valid.

Since Muller's discovery of the effect of X-rays several biologists were of the opinion that the spontaneous mutations are caused by the natural shortwave radiation, including the cosmic rays, omnipresent in nature. Their arguments would necessarily stand or fall on the nature of the dosage effect curve at very low radiation intensities. The calculations of Muller and Mott-Smith (1930), of Timofeeff-Ressovsky (1930) and of Efroimson (1931) showed that natural radiation is utterly inadequate to explain the spontaneous mutation rate, if the linear relation of mutation frequency to dosage of radiation holds true for very low intensities. Muller and Mott-Smith calculate that the radiation received by the flies in the entire period of their reproductive generation (from the germ cell of the

parent to the germ cell of the offspring) is 1300 times too low to explain their spontaneous mutation rate on the principle of a linear relation. Some biologists had thought, however, that radiation might actually be more effective at low intensities. The data obtained by us gives no indication of such an effect, any more than of a falling off of effectiveness at lower intensities. And we are now even more justified than before in extrapolating the dosage effect curve to even the lowest intensities of radiation. This would leave no chance for natural radiation to have produced an appreciable proportion of the natural mutation in Drosophila.

But the reproductive generation in the flies is ordinarily only a few weeks. In this connection it is of interest to compare the duration of the reproductive generation of the flies to that of human beings, which can roughly be taken as thirty years. The ratio of thirty years to two weeks is about 750, i.e., of the order of one thousand. The spontaneous mutation rate is, of course, not known in the case of human beings except for a few genes (Haldane, 1935), but here it is of the same order

as for the Drosophila genes studied. The mutability per unit of time in slow breeding animals is probably much lower than in Drosophila. Had it not been so all their chromosomes would be full of lethals within a few generations (Muller & Altenburg, 1919). Thus, although the natural radiation present is inadequate to explain any considerable part of the mutation rate in flies, this might not be true in the case of slow breeding animals such as human beings. This should not, however, be taken as a denial of the existence of other conditions than radiation influencing the mutation frequency in such organisms.

A medical consequence of the validity of the Bunsen-Roscoe law is that any person exposed to radiation, no matter what the intensity is, will tend to transmit genetic changes to his progeny, their frequency being in direct proportion to the total dosage of exposure. Account must however be taken, in such a calculation, of the fact that the mutations are produced in mature spermatozoa at several times the rate obtaining in ordinary cells, and that the duration of this stage is usually not

more than several weeks.

The low intensities used in this experiment was, of course, much above what is defined as the tolerance dose. But if we may make this generalisation that a single mutation is the result of a single 'successful' ion we must conclude that it is essential for radialogists to guard the reproductive organs of themselves and their patients against irradiation even of very low intensities on account of the danger of the genetic effects.

In the case of other biological effects of X-rays than the genetic effects, we find that dosages of radiation below certain intensities are not as effective as the same dosages at higher intensities. In these cases then, we are evidently dealing with more complicated effects, in which considerations (a), (b) or (c) (page 37) apply, as well as considerations of the variability of the material.

## SUMMARY.

1. Drosophila sperms were irradiated in the body of impregnated females with 70 milligrams of radium continuously for a period of 720 hours at two dosages of 2000 and 400r with an intensity of 1/20 and 1/100r per minute, respectively.

2. The frequency of the sex-linked lethals produced was found to be directly proportional to the dosage independent of the intensity of irradiation.

3. To compare the results obtained at lower intensities, another group of impregnated females were radiated with an intensity of 4/5r per min. giving a total dosage of 2000r in 45 hours. The mutation rate was found to be independent of the duration and intensity of treatment and dependent only on the total dosage applied.

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# PART - II.

## Translocation.

# INTRODUCTION.

Many observations of the past decade have combined to show that rearrangements of sections of chromosomes are of fairly common occurrence in population of Drosophila and of various other oganism, and that they also occur commonly as differentia of related species and even of races of the same species. There are, moreover, theoretical reasons for supposing some of them to play a role in the splitting of species in evolution, through their effects on processes of re-combination and others to be of evolutionary importance by leading to "repeats" of chromosome sections, whereby the number of genes becomes increased in evolution. Since the discovery of the phenomenon of the 'position effect' (Sturtevant, 1925) and its possible bearing on the gene changes associated with sectional re-arrangements (Muller, 1930) it is also evident that these re-arrangements may, like ordinary gene mutations, be subjected to direct negative or positive selection as a result of their phaenotypic effects, and thus too have an evolutionary value. Finally, the finding that there are minute re-arrangements which are difficult and

sometimes impossible to distinguish (Muller, Prokofyeva and Raffel, 1935) raises more sharply the question of what, if anything, is the essential difference between the two classes of phenomena and to what extent they may represent results of the workings of fundamentally the same mechanism of change.

All these considerations make it the more interesting to know what the mechanism is, whereby sectional rearrangements of chromosomes are brought about. Since the discovery that they are produced in high frequency by X-rays (Muller, 1927; Muller and Altenburg, 1930) it has become evident that one possible angle of attack on this problem is through a study of the conditions influencing their production by radiation even though it should be realised that the mechanism of their spontaneous occurrence may differ in important respects.

Until very recently, while many exact studies had been made of the conditions influencing the production of "gene-mutations" (as represented chiefly by sex-linked lethals) by radiation, very few studies had been made along the same lines with reference to the production of rearrangements.

We can approach the problem of the mechanism and the nature of the production of the chromosome rearrangements, indirectly, by inducing structural changes in the chromosomes and determining their frequencies quantitatively under varied conditions of radiation.

Dosage variation: Unlike the gene mutations, the dosage frequency relationship of gross chromosome rearrangements, (translocations, inversions, etc.) has been a matter of dispute. The earliest studies of Muller and Altenburg (1930) showed that the frequency increases with dose but the doses given were not exact enough to show whether or not the frequency was proportional to dose. Oliver (1930), working under Muller's direction, detected the chromosomal rearrangements which were associated with lethals and studied their relative frequencies at different dosages. His earlier data indicated that the frequency changed more rapidly than the dosage, but on the basis of further date (1932) he was inclined to view the relationship as linear. It was admitted, however, that these data were insufficient for a decision. Soon after these more extensive studies of Muller and his collaborators

(work on deletions by Muller, Vogt and Koerner, data of 1932-1933, referred to by Muller, 1936, 1937, 1938, and by Belgovsky and Muller, 1938, on translocations by Belgovsky, 1937, and by Muller, Makki and Sidky, 1938, and on inversions by Berg, Pauslin and Borisoff 1935, unpublished,) showed the frequency of gross re-arrangements to vary as the 3/2 power of the number of ions produced by X-radiation for the range of dosage between about 1000r and 4000r.

Khvostova and Gavrilova (1935), however, working under the direction of Dubinin, reported data giving a direct linear relationship between the dosage and the frequency of fourth chromosome interchange having a position effect on cubitus interruptus and they report having confirmed this later (1938), although at the same time finding the frequency of ordinary translocations to change more steeply than the dosage.

Heptner and Demedova (1936), also working under Dubinin, and studying by genetic methods the dosage frequency relationship of mutations involving individual loci and also of deletions, report that

there exists a direct linear proportionality between the dosage and the frequency of both of gene mutational changes and gross rearrangements (deletions), for doses of 1000r to 4000r, while at 6000r the frequency of both types of changes rises disproportionately.

Catcheside (1938), working at Pasadena, studied the frequency of induced structural changes of chromosomes observed in the salivary gland cells of $F_1$ female larvae of Drosophila melanogaster raised from X-rayed males and concluded that there exists a direct linear proportionality between the rearrangements and the dosage (between 1000 and 4000r). Similar results are reported by Buzzati-traverso (D.I.S. 1939) working under Timofeff-Ressovsky.

Opposed to the results of the above workers and agreeing with those of Muller and his collaborators, are the recent results of Sax (1938), working on Tradescantia microspores. He finds a geometric increase in the frequency of chromosome abberations with increased dosage, the frequency varying as the 3/2 power of the dosage. Bauer, Demerec and Kaufmann (1938) have very recently obtained somewhat similar results to those in their Drosophila material, using

methods like those of Catcheside. They believe, however, that their curve of results would fit the expectation for proportionality except at one point.

Two hypotheses have been put forward regarding the mechanism of the production of these aberrations, and as Muller (1932) has pointed out, a study of the dosage frequency relations should throw light upon which of these hypotheses is correct. A direct linear relationship demands that the production of any one gross rearrangement is caused by a single ionization while an exponential relationship indicates the concurrence of two or more ionizations in its production. The first of these relationships would be expected if contact or close proximity between the chromatids at the given point was a necessary factor in their breakage and re-union, while the latter would be expected if the chromatids first became broken, regardless of their contact or propinquity.

Of these two alternative mechanisms first mentioned by Muller (see Painter and Muller, 1929) the former was, independently, proposed and elaborated by Serebrovsky (1929), as an explanation

of all mutations and rearrangements, and was accepted by Dubinin (1930) who specially showed its application in the same deletions. Muller at first (1932) was inclined towards it, on the basis of Oliver's results that seemed to indicate a proportional relationship. He still adheres to this interpretation for most cases of minute rearrangements. According to this the two breaks involved in a rearrangement are not independent, but one break somehow acts to induce another one, or, more likely, both are due to a common cause, the effect of which spreads. This interpretation essentially represents the rearrangements in question as virtually crossing-over except that it occurs between non-homologous regions at a time other than synapsis.

The other alternative, that is, the breakage hypothesis, was early championed by Stadler (1931a; 1931b; 1932), and has been advocated by Muller also, since he obtained his results on the deletion frequency dosage relations in 1933, as an explanation of at least a part of the gross rearrangements. According to this view, the breaks are independently

caused by separate events and the re-union, which is subsequent, might or might not be limited in space and time.

Variation in the wave length of radiation :

It is now clear that short wave radiations ranging from the region of ultraviolet (Altenburg, 1933 ) to gamma rays of radium (Hanson & Heys, 1929) are all effective in the production of mutation. The effect for rays as short as the longest X-rays or shorter, was found to vary with the total number of ionizations, independently of the wave length.

Stadler and Uber (1938), working on maize, and Muller and Mackenzie (1939) on Drosophila, compared the frequency of mutations to rearrangements, when radiated with ultra violet. Using doses which led to a frequency of mutations high enough to have been accompanied by a noteworthy production of rearrangements, had the agent been X-rays, they found no rearrangements at all (except, apparently, in the case of maize, some terminal deficiencies). It was concluded from the results obtained that there is a difference in the genetic action of ultra violet and X-rays, which in turn indicates the

existence of a difference in the mechanism whereby the 'point changes' leading to gene mutations and to gross rearrangements, respectively, are produced.

No work has yet been done, however, on the comparison of the frequency of chromosome rearrangements when similar dosages are applied for the region of the spectrum extending from X-rays to gamma rays.

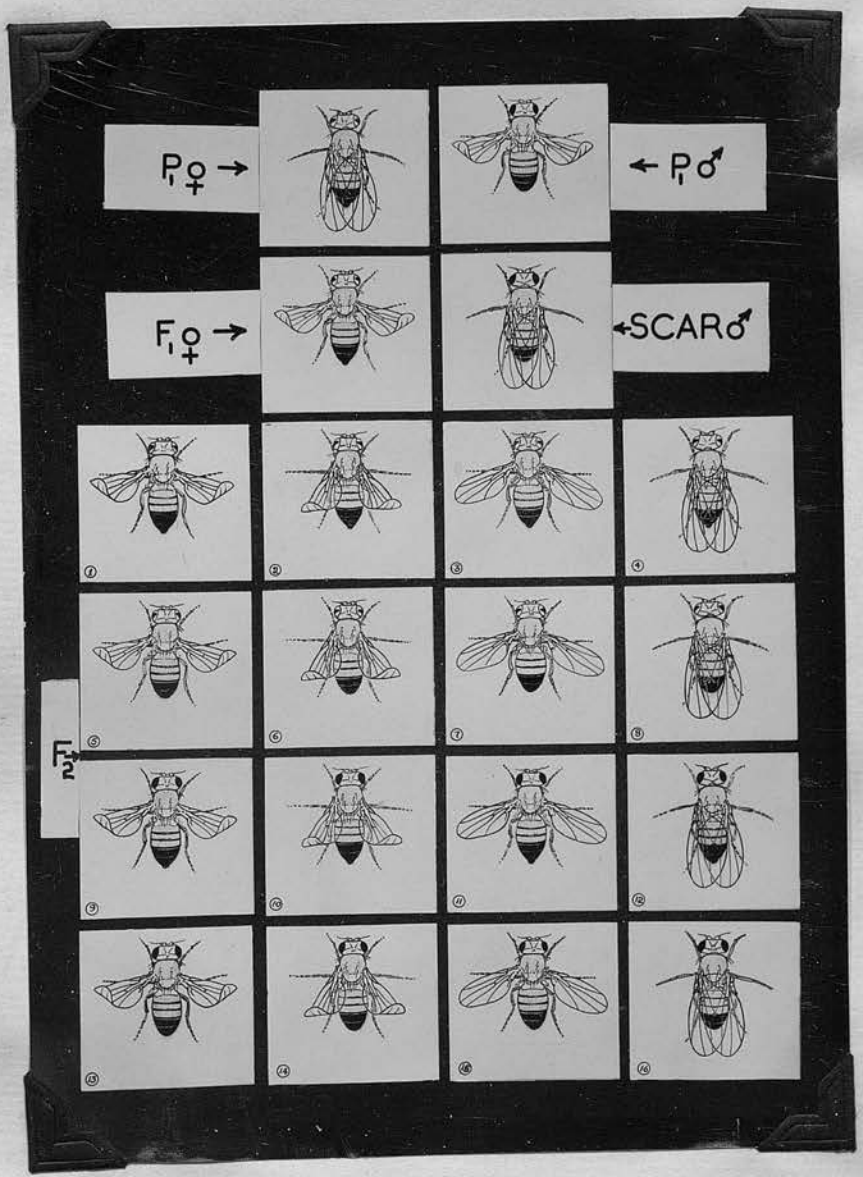
Time-intensity Factor:

The time-intensity factor in relation to gene mutation and its significance in this regard has been dealt with in our parallel paper. No work, as yet, has been done along this line in Drosophila so far as gross rearrangements of chromosomes are concerned. Studies of this matter may be expected to give valuable information about the mechanism of the production of rearrangements and about the properties of the chromosomes themselves.

Since the completion of our own work, Sax (1939) has reported evidence of the occurrence of a lesser number of chromosome breakages in Tradescantia microspores, at a lower intensity of radiation, as compared with the same dosage at a higher intensity.

Accepting the breakage theory, he explains the effect of time factor thus: "If the radiation is given slowly a break in one chromosome may heal before another break occurs in an adjacent chromosome. The second break also heals and no visible aberations appear at metaphase. When the radiation is intense two adjacent breaks may occur within the critical time period, so that fusions can take place between broken ends of different chromosomes." He also states that the broken ends of chromosomes may result in an unstable condition, in which they retain their capacity to fuse with the broken ends of different chromosomes for about an hour. These extremely interesting facts are important from the standpoint of the effects of radiation (both genetical and general biological). It is, however, very important to test these questions on Drosophila material also, not only because the hereditary material may be differnetly constituted in the two groups, one belonging to the plant the other to the animal kingdom, but also because it is possible, in Drosophila, to study cells (spermatozoa) in which the chromosomes are in a special physiological condition.

The present investigation, then, was undertaken to test the points raised above in our Drosophila material.

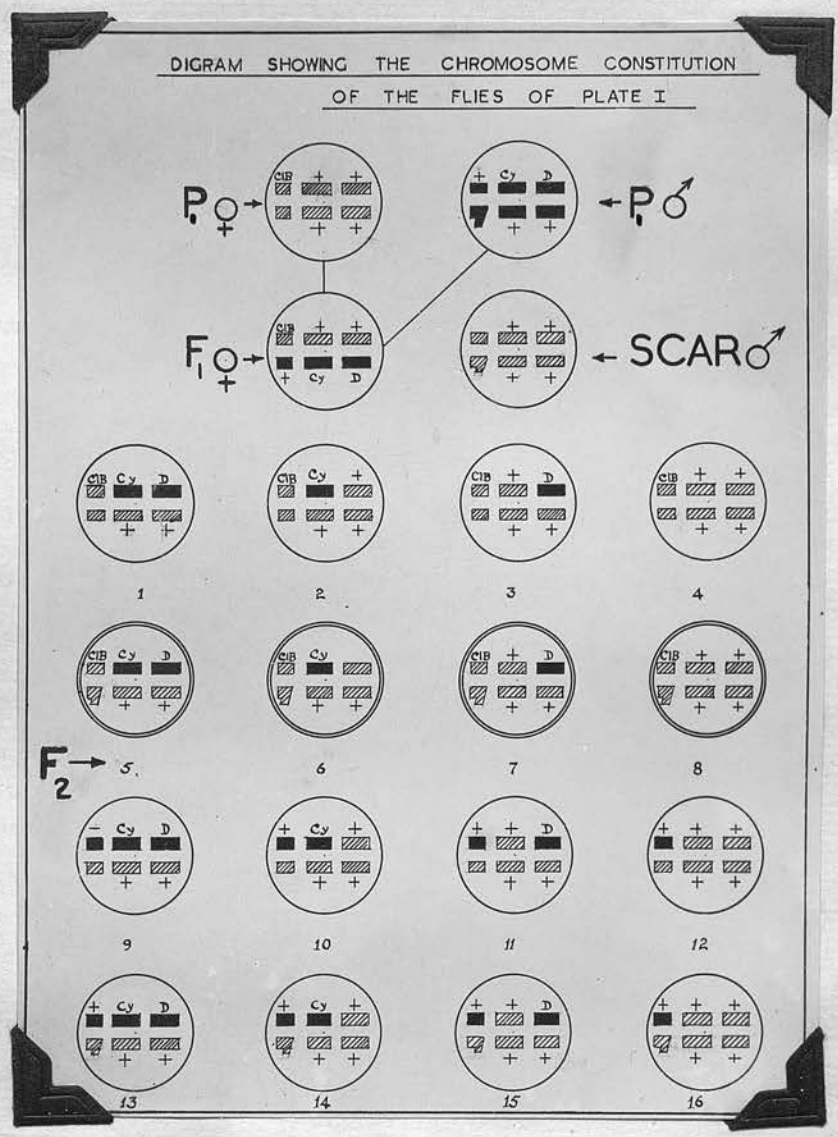

P1 ♀ →
← P1 ♂
F1 ♀ →
← SCAR ♂
F2 →
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

## Material and Method.

The method of detecting translocations and the genetic reasoning behind it is essentially simple. Advantage is being taken of the fact that the change in the normal "balance" in the gene contents of the gametes by way of duplication or deficiency of a section of a chromosome tends to produce phynotypic abnormalities or, in the case of gross change of "balance", a full lethal effect on the zygote.

Cy D males were allowed to inseminate ClB virgin females. The inseminated females were then rayed for 720 hours before allowing them to breed. The $F_1$ virgin females of the above cross having the following constitution $\frac{ClB}{+}\ \frac{+}{Cy}\ \frac{+}{D}$ were bred separatlely in small vials having one male and one female in each vial. "Scar" males were used for these pair matings.

The chromosome constitution of the $F_2$ flies (Pl.I) are shown in plate II, in which the distribution of the treated chromosomes of the sperms are shown with heavy lines. It is evident from the distribution of the chromosomes that a translocation involv-


DIGRAM SHOWING THE CHROMOSOME CONSTITUTION
OF THE FLIES OF PLATE I
P ♀
P ♂
F1 ♀
SCAR ♂
CtB
Cy
D
F2
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

ing any considerable transfer between any two chromosomes of the treated sperms (and the great majority of translocations do involve considerable transfer) can be easily detected by the apparent linkage of the markers situated in the two different chromosomes involved in the translocation. For, to produce normal viable zygotes, when fertilised by normal sperm, the eggs produced by the $F_1$ females must contain either both the chromosomes involved in the translocation or neither of them, to keep the normal genic "balance". All the other eggs which have received at the reduction division only one of the affected chromosomes involving the translocation die, either due to the deficiency or the duplication or (more usually) both, of sections of chromosomes.

The mothers of the $F_2$ families produce normally sixteen types of progeny (see plate I). Any ordinary translocation involving transfer between two or all of the major chromosomes - the X, second and third - can be easily recognised by the absence of certain definite recombination classes of these progeny due to translocation. The chromosomes containing those

markers which fail to show recombination with each other must be the ones which have undergone translocation (see table I).

The examination of the $F_2$ flies can be carried out without removing the flies from the vials. Because of the conspicuousness of the markers, here used, examination of these vials can be done without etherising the flies at all. A Zeiss binocular lens with head band giving 5 times magnification was found convenient for examination of vials.

In spite of all the advantages of using this method, it was found difficult to follow under the conditions of this experiment, because the number of $F_1$ females of the right kind for showing in their progeny both translocation and mutation, were only 1/16th. of all the $F_1$ zygotes. This, combined with the fact that the females had to be kept so long before breeding, and that both female and male germ cells were irradiated, made it too difficult to obtain enough $F_1$ for the tests, and added to this was the difficulty that the $F_1$ females on this method had to be strictly virgin for the detection of the translocations in question.

TABLE I.

Showing the presence and absence of different classes of $F_2$ progeny for the detection of translocations.

| Translocation between | Presence of these classes. | | | Absence of these classes. | | |
|---|---|---|---|---|---|---|
| | Males | Females | | Males | Females | |
| | Non B | Bar | Non B | Non B | Bar | Non B |
| X-Cy | NonBCyD-(13)<br>NonBCy-(14) | BD-(3)<br>B -(4) | NonBCyD-(9)<br>NonBCy-(10) | NonBD-(15)<br>Non B not Cy not D(16) | BCyD-(1)<br>BCy-(2) | NonBD-(11)<br>Non B not Cy not D-(12) |
| X-D | NonBCyD-(13)<br>NonBD - (15) | BCy-(2)<br>B -(4) | NonBCyD(9)<br>NonBD-(11) | NonBCy-(14)<br>Non B not Cy not D(16) | BCy-(1) D<br>BD -(3) | NonBCy-(10)<br>Mon B not Cy not D -(12) |
| Cy-D | NonBCyD-(13)<br>Non B not Cy not D-(16) | BCyD-(1)<br>B not Cy not D-(4) | NonBCyD-(9)<br>Non B not Cy not D-(12) | NonBCy-(14)<br>Non BD-(15) | BCy-(2)<br>BD -(3) | NonBCy-(10)<br>Non BD-(11) |

N.B. Numbers in parenthesis correspond to the numbers indicated in the plate showing the different types of $F_2$ flies.

Method of detecting translocations with recessive markers :

While the method described above will be found very convenient in ordinary work when only the males are irradiated and the females are bred immediately after mating, it was decided after considerable trial to change the technique for the purpose of the present investigation and use different stocks for detecting translocation and mutation separately. The results regarding lethals obtained in the trials of the first method have been incorporated in our present report, since it is explained that all the Bar $F_1$ females could be used for detection of lethals. But too few certainly virgins Bar Cy D $F_1$ females were obtained to make the data on translocations reliable, so has been omitted. We may now describe the method used thereafter for detecting mutations and translocations separately. bw e ey homozygous virgin females from the stock of that kind were crossed with wild-type males of Samarkand. The $F_1$ males of this cross (phenotypically wild type) were back crossed individually in vials to bw e ey homozygous virgin females from the stock. The

distribution of the markers was followed in each of the $F_2$ families for the detection of translocations. The $F_1$ males used for testing the translocations had received the Y- chromosome from the treated sperms. Thus, the translocations between the Y and autosomes were detected in this case instead of those between the X and the autosomes, as on the preceding method. It was impractical to make the examination of the flies within the vials. The examination of the progeny ($F_2$) was more difficult in this case because the markers used were less conspicuous. All the vials were therefore etherised by the mass method of Altenburg and every group of $F_2$ was removed from its vial and examined separately under the binocular microscope. The method of detection involved, as before, examination for the presence of recombinations between each two markers. No new table is given to represent the expected progeny here because this would only involve the substitution of the present markers for those previously used in the corresponding chromosomes.

Table II shows the different groups of experimental flies under comparison.

TABLE II.

Showing the different groups of experimental flies for the detection of translocation.

| Groups | Total dosage | Period of irradiation | Intensity per min. |
|---|---|---|---|
| A. High treated group | 2000r | 720 hours | 1/20r |
| B. Low treated group | 400r | 720 hours | 1/100r |
| C. Concentrated (2000r) | (2000r) 1300 | 45 hours | 4/5r |
| D. Concentrated (400r) | (400r) 260 | 9 hours | 4/5r |

## RESULTS.

Three series of experiments were carried out for the detection of translocation by using recessive markers. These three series correspond to the last three series (series VI, VII and VIII) of our previous paper, total doses of 2000r and 400r, respectively, being given to each of two groups of impregnated females over a period of 30 days in each of the three series.

Tables III & IV show the numbers and kinds of translocations obtained in each group in each series. The differences in the frequency of translocations between the different series are not significant. In all, 21 translocations were obtained in a total of 469 $F_1$ males tested in the high treated group - a percentage of 4.47 ± 0.96. Only 1 translocation was obtained in 1208 males in the low treated group - a percentage of 0.08 ± 0.08 (see Table V ).

1. Validity of Bunsen-Roscoe law :

Table V shows the comparison between the results mentioned above and those of the two "concentrated"

N.B. Throughout the present paper the values shown following the symbol ± denote standard errors, not probable errors.

TABLE III.

Showing the numbers and kinds of translocation obtained in the different series.

| Series | High treated group | | | Low treated group | | |
|---|---|---|---|---|---|---|
| | No. of $F_1$ males tested | No. of translocations obtained | Kinds of translocations | No. of $F_1$ females tested | No. of translocations obtained | Kinds of translocations |
| I | 131 | 7 | II & III | 602 | 1 | II & III |
| II | 243 | 9 | (1 - Y & II<br>(8 II & III | 486 | 0 | - |
| III | 95 | 5 | II & III | 120 | 0 | - |
| Total | 469 | 21 | | 1208 | 1 | - |

TABLE IV.

Showing the numbers and kinds of translocation obtained in the Concentrated groups.

| Dose | No. of $F_1$ males tested | No. of translocations obtained | Remarks |
|---|---|---|---|
| 2000r | 473 | 19 | 1 - II & IV<br>2 - Y & II<br>16 - II & III |
| 400r | 601 | 1 | II & III |

TABLE V.

Showing the validity of the Bunsen-Roscoe law in the production of translocation.

| Diluted treatment | | | | | Concentrated treatment | | | | Time intensity range | |
|---|---|---|---|---|---|---|---|---|---|---|
| Dosage | Intensity in r/min | No of $F_1$ flies tested | No of translocations obtained | Frequency in % (Fd) | Intensity in r/min. | No of $F_1$ flies tested | No of translocations obtained | Frequency in% (Fc) | | Difference $F_d \sim F_c$ |
| 2000r | 0.05 | 469 | 21 | 4.47±0.96 | 0.8 | 473 | 19 | 4.01±0.90 | 16 | 0.46±1.32 |
| 400r | 0.01 | 1208 | 1 | 0.08±0.08 | 0.8 | 601 | 1 | 0.17±0.17 | 80 | 0.09±1.8 |

groups given total dosages of 2000r in 45 hours and 400r in 9 hours, respectively, at an intensity 16 times as high as that of the high treated group reported above. In the concentrated group we obtained 19 translocations in 473 $F_1$ males and 1 translocation in 601 males at the dosages of 2000r and 400r, respectively.

The difference between the frequencies of translocation at the dosage of 2000r at the two intensities is 0.46 $\pm$ 1.32% and therefore insignificant. Similarly, at the dosage of 400r, the difference is 0.09 $\pm$ 1.8%.

In the above two high dosage groups where the intensity of treatment was varied, the time was varied inversely, so as to keep the total dosages constant. The same held true for the two low-dosage groups. As we find no significant difference between the effects at the two intensities in either case we can conclude on the basis of our data that the Bunsen-Roscoe law holds true in the production of translocations by gamma rays in Drosophila sperms.

2. Frequency-dosage relation :

Table VI shows the frequency-dosage relation in the production of translocations at the two

TABLE VI.

Showing frequency-dosage relation in the production of translocation.

| Group | High treated, 2000r | | | Low treated, 400r | | |
|---|---|---|---|---|---|---|
| | No. of $F_1$ males tested | Translocation | % of translocation | No. of $F_1$ males tested | Translocation | % of translocations |
| Concentrated treatment | 469 | 21 | | 601 | 1 | |
| Diluted treatment | 473 | 19 | | 1208 | 1 | |
| Total | 942 | 40 | $4.25 \pm 0.65$ | 1809 | 2 | $0.11 \pm 0.07$ |

| Doses | % of translocations obtained | % expected when the exponent is 1.5 | Difference ( D - F) | % expected when the exponent is 2 | Difference ($D_1$ - F) |
|---|---|---|---|---|---|
| 2000r | $4.25 \pm 0.65$ | | | | |
| 400r | $0.11 \pm 0.07$ (F) | $0.38 \pm 0.06$ (D) | $0.27 \pm 0.09$ | $0.17 \pm 0.03$ ($D_1$) | $0.06 \pm 0.07$ |

dosages studied by us. For this purpose we have added together the results of our diluted and concentrated groups. 40 translocations were obtained at 2000r in 942 $F_1$ males tested, which gives a frequency of 4.25 ± 0.65%. In the group given one fifth this total dosage (i.e., 400r) we have 2 translocations in 1809 $F_1$ males, i.e., 0.11 ± 0.07% (see table VI). If we assume that the frequency of high treated to low treated varies between the dosage here used, as the 3/2 power of the dosage, i.e., as $5^{3/2}$ : 1, we should expect calculating from the results at 2000r, a frequency of 0.38 ± 0.06% at 400r; instead we find 0.11 ± .07%. The difference is 0.27 ± 0.09%. The ratio of the difference to its error is ca 3 times, which makes it very probable that the value of the exponent is really greater than 3/2. We can now calculate to see whether the value of the exponent is significantly different from 2 or not. If the exponent is 2 we expect the frequency at the low dosage, as calculated from the results of the high dosage, to be 0.17 ± 0.03%; instead we obtained 0.11 ± 0.07%. The difference here is

0.06 ± 0.07, which is clearly not significant.

Thus we can reasonably conclude from our data that the value of the exponent is significantly greater than 1.5 but not significantly different from 2. This is, as we shall see, just what we should expect on the breakage theory at such low total dosages, and this result stands in contrast to the approximately 1.5 exponential relationship expected on this theory, for higher total dosages than those here used and actually obtained by workers (Muller, Belgovsky, Sax, Sidky) employing such dosages.

3. Independence of wave length :

Tables VII & VIII show the comparison of our results obtained with gamma rays of radium (high dose, 2000r) with those of Makhijani obtained with similar dosages of X-rays (1500r). While calculating the percentages here given we restricted ourselves to the frequency of translocations between the IInd and the IIIrd chromosomes, since Makhijani's data show only this class.

Comparing our results of the frequency of

translocations at the dose of 2000r with those of Makhijani at 1500r we have assumed, in accordance with the previous results of Muller and his collaborators, that the value of the exponent is 1.5 between these dosages. If we take the percentage of lethal mutations (5.94 and 6.67%, see table VII) as the more accurate measures of the dose actually received, and use the gamma ray translocation results as the basis of calculation, we find that the X-rays, considering their lower dose, should have given 3.1 ± .6% of translocations, provided there there the differences in wave-length and in time-intensity were without influence. This calculated result is not significantly different from what we actually obtained in our gamma ray experiment (3.8 ± 0.6%). If on the other hand we take the dosimeter reading of the X-ray experiment and the dosage calculated on physical grounds for the gamma ray experiment as the measure of the dose, and again calculate the X-ray results, we find that X-rays should have produced 3.6 ± .4% of translocations, which again is not significantly different from our obtained figure of 3.8 ± 0.6%.

TABLE VII.

| Wave length | Dose | Percentages of sex linked lethals obtained | % of Translocations (II & III) |
|---|---|---|---|
| A. X-ray (Makhijani | 1500r | 5.94 ± 0.93 | 2.57 ± 0.3 |
| B. Gamma ray (Ray-Chaudhuri) | 2000r | 6.67 ± 0.85 | 3.8 ± 0.5 |

TABLE VIII.

Comparison of the frequency of translocations obtained by gamma rays (Ray-Chaudhuri) with those obtained by equal dose (2000r) in X-rays (Makhijani).

| Basis of Calculation | % translocations obtained by gamma rays $F_c$ | Expected percentage of translocations with X-rays $F_d$ | Difference $F_d$ $F_c$ |
|---|---|---|---|
| Calculared on the basis of dosimeter readings | 3.8 ± 0.6 | 3.6 ± 0.4 | 0.2 ± 0.7 |
| Calculated on the basis of lethal mutation frequency | 3.8 ± 0.6 | 3.1 ± 0.6 | 0.7 ± 0.8 |

Thus comparing the results both ways, viz : (1) taking lethal mutation rate as the measure of the dose and (2) taking dosimeter as the correct measure of the dose, we find that X-rays and gamma rays produce sensibly the same frequency of translocation at a diven dosage. In other words, wavelengths within the very considerable region of the spectrum ranging from X-rays to gamma rays have no differential effect on the production of gross rearrangements.

The above comparison also allows us to extend our conclusions regarding the Bunsen-Roscoe law, to a considerably greater intensity-time range than before. For the difference existing between Makhijani's X-ray irradiation of 100r/min and our dilute low-treated gamma ray lot of $\frac{1}{100}$r/min represents a range of 10,000 times, i.e., orders of magnitude.

## DISCUSSION.

Whatever may be the mechanism of the production of chromosome rearrangements, it is clear from our results that, similarly to gene mutations, they are produced in spermztozoa independently of the time-distribution of the ionization, and also independently of such differences in space distribution as characterise X as compared with gamma rays.

It is generally believed that the primary causes of gene mutations and gene rearrangements are in some way inter-related. This view finds strong support if we consider all the implications of the validity of the Bunsen-Roscoe law, and of the independence of the wave length, found both in relation to gene mutations and, now, in relation to chromosome rearrangements.

Accepting the breakage theory on the basis of the exponential relation of rearrangement frequency to ionization frequency, we may reason further, as follows. In producing the rearrangements, individual ionizations probably act by producing individual breakages of chromosomes, but these breakages cannot

result in rearrangements until at least two breakages have occurred, allowing the re-union of broken pieces in a different arrangement than before. In view of the independence of the rearrangements from the time distribution of the ionizations, even when the latter are scattered over the period of a month, we must conclude that these broken ends remain as such until the sperms enter the eggs. Then, at the time when the nuclear membrane disappears and the movements of the chromosomes become comparatively free, the broken ends do actually get a chance to unite with one another.

We may infer that previously to this, in the spermztozoa, some condition obtained which made the broken ends unable to unite, even when they accidentally (as through Brownian movement) came into contact, whereas after fertilization they became capable of union. For it was obvious that, due to the spatial propinquity of the broken ends in the sperms, there was a far greater chance for the broken ends to re-unite in the 'old' order, provided they were able to unite while in the spermtozoa at all. Those that did this would fail to give rise

to rearrangements. Now those breakages which were produced in our experiment during the earlier portion of the month-long period of irradiation would have had this supposed chance of re-uniting. Consequently, if this possibility really existed, we should reasonably expect a difference between the frequency of translocations in our two groups, involving diluted and concentrated treatments, respectively. And there would be a similar difference between the results of our diluted radium treatments and the results of the experiments of others in which an equivalent dosage of X-rays was given in concentrated form, followed by immediate breeding of the treated flies. But when these comparisons are made no such effect is to be detected.

The latter comparison, involving our dilute-series results and those of X-ray experiments, was made by using for comparison with our results those obtained at the same time in our laboratory by Makhijani (as yet unpublished), since he used a total dose not very different from ours, as shown both by the dosimeter readings (1500r) and by the percent of

lethals obtained. As shown in the preceding section, the difference between his results and ours is not significant, when by the use of the 3/2 frequency-dosage relation we calculate the per cent of translocations which he would have obtained at our dose.

It should in addition be mentioned that Makhijani made the question of whether or not re-union of broken ends occurs within the spermztozoa the subject of a special study, by having, besides the above series in which the X-rayed flies bred, immediately, another in which the mature spermztozoa, after the same treatment, were held for a month in the female at 8°C, before fertilization was allowed. As shown in his paper, the results from this series were sensibly like those of the former series, and so were those from a third series, in which the dose was "fractionated" during the course of a month, during which the mature spermztozoa were held. Thus his data as well as ours show clearly that there are no detectable differences between the results in the different groups in which there was more or less chance given for re-union of broken ends into the old combinations to occur within the spermatozoa.

The assumption that no re-union really happens until the sperms get into the eggs is, however, not necessary if we accept the "contact" mechanism for the production of the rearrangements. In that case, the chromosomes involved in a translocation would break at their junction or point of propinquity, due to a common cause (a single ionization), and the re-union, giving either an inviable or a viable combination (including in the latter case the possibility of restitution), could conceivably take place in the sperms. The timing of the treatment would in that case not be expected to affect the chance of rearrangement occurring.

We are, in fact, forced to assume some such mechanism to be operative (though the reunion need not necessarily take place in the spermztozoon stage) in the cases of minute re-arrangements, including small deletions (amongst which some of the lethals produced by X-rays are numbered). For these deletions and other minute rearrangements, which necessarily involve two breakages, though comparatively near to each other, are definitely proportional in frequency to the dosage. as recent results

obtained by Belgovsky and in our laboratory show (see Muller, 1937, 1938; Muller, Makki & Sidky 1938), and as Sax (1938) also has found.

The difficulty in accepting the "contact" mechanism in the case of the gross rearrangements lies in the nature of the rearrangement frequency-dosage curve. The argument is as follows: Suppose we have a number of particles arranged in a number of different chains (chromosomes); we can plot the curves relating to the frequency of incidence of the causative events (ionizations) and the frequency of the final changes (rearrangements). Now, if only one causative event is necessary to produce a final change, this curve will be linear (at least until the "saturation effect" causes it to rise still less steeply), but if more than one is necessary an exponential curve would result.

When Muller (1932) regarded the breakage hypothesis as the less likely one, it was because the available evidence at that time was taken as indicating a linear relationship, but he took up the breakage hypothesis as probably being true, in part at least, when his and Belgovsky's later results (1933 et seq)

showed an exponential relation. For a time it seemed as though both processes might be going on in the production of gross rearrangements, in view of the relation being between that of simple proportionality and that of the frequency varying as the square of the dosage. It was realised that viability effects, varying with dosage, might play a role in the reduction of the exponent below the square, but such effects were not quantitatively estimated. This was, however, done by Haldane (1935, unpublished), whose calculations showed that the breakage hypothesis could give a less-than-square relationship, owing to the higher viabilities of the multiple-break classes that are found in higher frequency at higher dosages. These higher viabilities are due to the fact that when there are more breaks there are relatively more chances for dicentric and acentric chromosomes to be formed. And S t a d l e r - who had from the beginning advocated the breakage hypothesis (1931a, 1932) - made a similar suggestion, pointing out that with a farily high frequency of breakages per cell, a part of the curve would even approximate to a

straight line.

Catcheside (1938), has made provisional calculations and tables of the results to be expected, and discusses the form of the curve of observed translocation frequency according to the two different hypotheses, separately. While not taking a dicided stand he leans towards the contact hypothesis although, as he points out, his own results are not dicisive in either direction. In calculating the results for the breakage hypothesis he has assumed (1) that breakage is at random, (2) that reunion is at random, and (3) that there is never more than one break per chromosome. While (2) is probably and (3) is certainly untrue, the calculations nevertheless show what trend the results would have, and it becomes clear that, for doses giving the translocation frequencies usually dealt with, the exponent of the dosage to which the frequency was proportional would be considerably below 2, though above 1, and would in fact be in the neighbourhood of 1.5, as has been observed in the experiments of Muller and collaborators, previously cited, on Drosophila sperm, and recently by Sax (op. cit.) on plant material. An

extension of Catcheside's tables to lower doses (lower breakage frequencies) than those considered by him would further show (though he did not point this out) that the exponent in question would increase, approaching 2, as the dose diminished.

On the other hand, Catcheside concludes that, on the contact theory, the frequency dosage curve would be "slightly sigmoid", but with the lower portions nearly linear. In fact, it would not be sigmoid, but would increasingly approach the straight line relationship (exponent 1) with decreasing dose, while with greater dose the curve would become somewhat convex, the exponent dropping below 1. As it could never rise above 1, the observed relations would seem to rule out the contact hypothesis.

Our own data not only confirm the data of Muller et al and of Sax in showing an exponent greater than 1, but give some evidence of the further feature of the breakage curve noted above, namely, that at low doses the exponent even rises above 1.5, approaching 2. In other words, it is probable that in the range of dosage studied by us the frequency of transloca-

tion varies more nearly as the square of the dosage than it does at the higher dosages more often investigated, just as would be expected on the breakage theory. And it certainly does not vary more nearly as the first power of the dosage, as would be expected on the view that the postulated "contact" mechanism was responsible for some appreciable part of the results.

To be sure, Makhijani's data on low doses give an exponent of almost exactly 1.5 (see his parallel paper). But whereas his results could well be regarded as accidental statistical deviation from a relation in which the exponent were considerably above 1.5, ours could not well be regarded as a deviation from a relation in which it was only 1.5. Thus, when both the experiments are taken together, it still remains probable that at low doses the exponent approaches 2, as expected on the breakage theory.

The success of the irradiation treatment of cancer lies in the efficacy of X and gamma rays in causing the death of the rapidly dividing cells that constitute the cancer tissue. It is evident that

these short-wave radiations must act in the cells, in part at least, by fragmenting the chromosomes, with the resultant production of acentric and dicentric recombinations that are mechanically unfit to divide normally, and also of combinations that lead to genic unbalance. These effects would naturally become manifested more rapidly in the case of cancer cells, on account of their greater rapidity of division, than in the case of the normal cells surrounding them.

As previously noted, the broken ends of chromosomes in the sperm cells of Drosophila are for some reason or other not able to unite until the time of fertilization, a fact which made it possible for us to show the applicability of the Bunsen-Roscoe law in the production of chromosome breaks. This condition in the sperm cells is, however, to be regarded as a special case. Thus Sax (1939) in his Tradescantia material found a lesser number of rearrangements when a given dose of X-rays was applied at a lower intensity. He interprets this as due to the fact that re-unions may take place

within the period of irradiation. The more prolonged the latter is, the more chance is given for re-union of broken ends previously together (restitution) to occur, before the later breakages take place, which would have provided other broken ends, for recombination with those caused by the earlier breakages. Evidently in material like this one cannot so readily prove the validity of the Bunsen-Roscoe law in the production of chromosome breakages.

In the light of the considerations just discussed, the findings that chromosome breakages are produced with equal final frequency at low and high intensity, given the same total dosage, and also that they are independent of the wave length of radiation, might have an important bearing on the treatment of cancer. For the breakages and the re-unions of chromosomes are two separate processes and both occur in a more or less random manner (subject to certain qualifications which we need not consider here). Now a single ionization causing a break in a chromosome in the prophase nucleus, when the chromosome is already split can produce four broken ends (Sax, 1938) and their re-union at random will produce,

chromosome ends have a better chance to undergo restitution in the resting nucleus than when subjected to the movements attendant upon mitosis. It is also likely that chromosomes in the relatively condensed condition in which they are during mitosis resemble the condensed chromosomes of spermatozoa in being for some reason more subject to breakage and mutational changes.

Thus radiation of relatively low intensity should produce a sufficient quantity of cell lethals to kill or interfere with the proliferation of many of the cancer cells, while not sensibly affecting the normal tissue. The determination of what dosage and intensity of radiation would be best for a successful treatment cannot, however, be made without experimentation on tissues of the type in question.

## SUMMARY.

Two lots of mature spermatozoa of Drosophila melanogaster (wild type), present in inseminated females of the bw e ey stock, were irradiated with gamma rays of radium for a period of one month (720 hours). They were given total doses of ca2000r and ca400r, at intensities of 1/20r and 1/100r per minute, respectively, and the frequencies of gross rearrangements (translocations) in the sperms of the two lots were determined. Two other lots of sperm were given the above total dosages at an intensity of ca4/5r min, the period of radiation being in this case 45 hours for 2000r and 9 hours for 400r. The results obtained are :-

1. The frequency of translocations in Drosophila sperms is independent of either the intensity or the time distribution of the dosage and is dependent only on the total dosage, thus following the Bunsen-Roscoe law.
2. The data on the frequency of translocations at the two different dosages shows an exponential relationship. The frequency varies more

at a rate of somewhat greater than the 3/2 power of the dosage, as is expected for these low doses on the breakage theory of the formation of gross chromosome rearrangements.

3. A comparison of the translocation frequencies obtained by Makhijani working in our laboratory with similar total dosages of X-rays (as measured both by dosimeter readings and lethal percentages) with the results of the present work shows that the frequency of translocation is independent of the wave length of radiation for the region of the spectrum extending from X-rays to gamma rays. It is also independent of the time-intensity factor, i.e., it follows the Bunsen-Roscoe law, over a time-intensity range of 10,000X.

4. The possible bearing of these findings have been discussed in relation to the treatment of cancer.

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